

Paris, le 27.10

1958

strains - as I +
acknowledged.
11/3

Dear Josh

This letter has been postponed more than is decent, particularly after your kind offer made in Stockholm! I won't give you a list of reasons, but only apologize for the delay.

I am slowly getting used to the idea that I might settle in the US, but when one is in his forties, and with a family, things are no longer simple - with me at least. At least, I am interested enough to tell Herlyn, who wrote recently, that I wanted to hear of how the situation develops in Madison.

The strains you asked for are being sent to you today. Here is some information about them - Both come originally from Alexander's lab. - Rd is an influenzae, Td a paraso.

Growth media are the same for both, except for the special growth factors (DPN for both strains, Hemin for Rd only). The stock-solutions of the factors are 250 µg/ml, added 1/100 (or a 10-30 times excess) - Hemin is dissolved as follows:

25 µg into 12.6 ml of $\frac{M}{2}$ Na_2HPO_4 - bring to a boil
+ 86 ml dist. water, + 1.6 ml of $\frac{M}{1}$ KH_2PO_4 . Autoclave.

DPN is dissolved in water, sterile filtered & kept cool.
The factor solutions are added to liquid media only - The solid medium is nutrient agar chocolate with whole hot with 1/50 vol. of defibrinated rabbit blood - and needs no further additions.

The basal liquid medium for growth can be any kind of broth, pH 7.3-7.5 - Periodically, growth may not start from a small inoculum and I add therefore routinely 0.2% final of serum albumin (Bovine, fraction V from Armour).

The strains are kept lyophilized: growth from a slant of chocolate agar suspended in ca. 5 ml of horse serum → enough suspension for 3-5 lyophilized tubes - kept thereafter at -10°C . - But when used routinely, the cultures are maintained on chocolate agar ~~and kept~~ at 37°C all the time, with one or two transfers a week.

Transformation experiments -

A) with Rd. The following medium gives \pm reproducible frequencies

($\frac{1}{300} - \frac{1}{1200}$) - For 1 liter, dist water.

[Difco Bactotryptone 20 gr
NaCl 5 gr (to be traditional)

Bring pH to ca 3.0 with conc HCl - & treat with Norite (2 gr.)

Filter on paper, adjust pH to 7.5 & autoclave.

Completed before use with $\frac{1}{20}$ vol of 4% albumin, pH 7.5 - and factors

Experiment.

Medium inoculated for 1×10^7 bact/ml, from growing culture (convenient to have this latter inoculated the night before, in water bath set to be turned on at 1 or 2 AM). Incubation unshaken - You watch the optical density; when it is such that you have 3×10^8 bact/ml, competence is good (doubling time ca 30 min - but don't go by the time)

DNA added, contact 5-10 min -

Now, in order to have expression you can -

- either dilute $\frac{1}{20}$ into fresh medium containing DNase (5-10⁸/ml) incubate ^{unshaken} 90 min & plate on choc. agar + Streptom (100⁸/ml final).
- or ^{Add DNase to undiluted culture,} take a sample, into a tube with 3ml of ^{melted} choc. agar, pour onto choc. agar plate, incubate 2-3 hrs 37° and then add 3ml of melted nutrient agar + Sm 1,000⁸/ml -

If an accurate measure of frequency is required, a ~~all~~ viable count is made ^{right} after DNase was added.

My best frequency has been $\frac{1}{100}$ - food gal says he gets as much as $\frac{1}{20}$ by aerating the culture, and then keeping it unshaken at 37° for 90 min. Although I did get a rise in frequency during unshaken incubation, I never got better than $\frac{1}{200}$ at the end, ~~because~~ as the initial frequency was so low.

② with Fid

I used it as receptor only sporadically & found the optimal conditions for competence to be different at 6 months intervals. I do not dare to recommend any particular medium or timing. You must try for yourself. Trying means

- try of different complex media (+ alb. + fact.) containing excess DNA (> 0.1⁸/ml final) - inoculate ~~at night~~ in the evening. Culture given opportunity to express the next morning, by either method described above, then challenged with Sm
- with best medium, establish curve of competence against

time by submitting cultures to successive ^{20 min} exposures to DNA as
it grows. — I never got better than 10^{-3} with F₂. — I have seen
competence occur as late as 3 hrs after growth has stopped,
with this bug —

Hope I am not forgetting anything essential. Anyhow please
write if anything goes wrong.

Best regards,

Yours very sincerely

Pierre

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